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In re application of:

Chatterjee, D.K.

Appl. No. 09/558,421

Filed: April 26, 2000

For: **Mutant DNA Polymerases and  
Uses Thereof**

Art Unit: 1652

Examiner: Rao, M.

Atty. Docket: 0942.3600003/RWE/BJD



### Declaration of Brian Schmidt

Honorable Commissioner of Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Brian Schmidt, do hereby declare and say:

1. THAT, I, Brian Schmidt, hold the degree of B.S. A recent copy of my Curriculum Vitae, accurately listing my scientific credentials and work experience, is attached hereto as Exhibit A.

2. THAT, since June 4, 2001, I have been employed by The National Institutes of Health. Prior to my current position, from November 25, 1985 to June 1, 2001, I was employed by Life Technologies, Inc. (LTI) (and now Invitrogen Corporation)<sup>1</sup>, the assignee of the above-captioned application, in the capacity of Staff Scientist. *See* Exhibit A.

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<sup>1</sup>Life Technologies, Inc. merged with Invitrogen Corporation on September 12, 2000, with Invitrogen Corporation being the surviving entity.

3. THAT, during my employment by LTI (and now Invitrogen Corporation), I worked under the supervision of Dr. Deb K. Chatterjee on a project involving the cloning, expression, and characterization of wild-type and mutant DNA polymerases.

4. THAT, I have reviewed my laboratory notebooks detailing my work on the project. Based on these laboratory notebook records and my recollection, the following activities involving my work, and relating to the DNA polymerase project, took place during the period from about October 16, 1994, until about September 8, 1995.

On or about October 13, 1994, I performed an experiment digesting a mutant *Tne* DNA with various restriction enzymes. This experiment was recorded on pages 49-50 of notebook 3884. A copy of thereof is attached as Exhibit 1.

On or about January 25, 1995, I outlined ideas for recloning the *Tne* polymerase gene fragment into M13 for mutagenesis. Additionally, I restricted pSport-*Tne* to confirm the identity of this plasmid. This activity was recorded on pages 51-52 of notebook 3884. A copy thereof is attached as Exhibit 2.

On or about January 26, 1995, I began an experiment to clone the *Tne* polymerase gene fragment into M13. He redigested pSport-*Tne* to confirm the identity of the plasmid. Preparative digests of pSport-*Tne* and M13mp19 were also performed. The fragments were ligated and used to transform DH10B host cells by electroporation. This experiment was recorded on pages 53-55 of notebook 3884. A copy thereof is attached as Exhibit 3.

On or about February 7, 1995, I began an experiment to clone a *Tne* gene fragment. Instead of using the *Bam*HI/*Sph*I digest, I used an *Sph*I digest in this experiment and would select for directionality using restriction mapping. pSport-*Tne* and M13mp19 were digested with restriction enzymes. The resulting restriction fragments were ligated together and used to transform *E. coli*. This experiment was recorded on pages 56-57 of notebook 3884. A copy thereof is attached as exhibit 4.

On or about February 8, 1995, I continued the experiment to clone a *Tne* gene fragment into M13. I digested new constructs ligated on February 7, 1995, with restriction endonucleases to select for the proper directionality of the insert. This experiment was recorded on pages 58-59 of notebook 3884. A copy thereof is attached as Exhibit 5.

On or about February 8, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone the *Tne* gene into M13. Plasmid was purified from the *Tne* clones obtained on February 8, 1995. This experiment was recorded on page 1 of notebook 3966. A copy thereof is attached as Exhibit 6.

On or about February 9, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. M13 ssDNA was purified, and new media and plates were made. Additionally, new cultures of CJ236 and the new *Tne* clones were started. This experiment was recorded on pages 2-5 of notebook 3966. A copy thereof is attached as Exhibit 7.

On or about February 16, 1995, I continued the experiment to clone a *Tne* polymerase gene fragment into M13 by assembling the restriction map of the T.nea/mp19 construct. This experiment was recorded on page 61 of notebook 3884. A copy thereof is attached as Exhibit 8.

On or about February 16, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* polymerase gene fragment, by determining a restriction map for the new *Tne* constructs. This experiment was recorded on pages 6-8 of notebook 3966. A copy thereof is attached as Exhibit 9.

On or about February 21, 1995, I requested the synthesis of a 26-mer mutant *Tne* oligonucleotide (designated oligo # 2899) and a 30-mer 5'-3' exonuclease mutant *Tne* oligonucleotide (designated oligo # 2900). Both oligos were synthesized by LTI personnel, with synthesis completed on February 24, 1995. This activity was recorded on page 23 of notebook 3964. A copy is attached as Exhibit 10.

On or about February 21, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. The *Tne* clones and M13 DNA were restricted for subsequent cloning. This experiment was recorded on pages 13-14 of notebook 3966. A copy thereof is attached as Exhibit 11.

On or about February 22-23, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. The

reactions from February 21, 1995 (*see* Exhibit P-4), were analyzed by gel electrophoresis. Restriction fragments were excised from the gel. M13 and the *Tne* polymerase gene fragment were ligated together. The products of the ligations were analyzed by agarose gel electrophoresis, and used to transform *E. coli*. This activity was recorded on pages 15-16 of notebook 3966. A copy is attached as Exhibit 12.

On or about February 28, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. The identities of pTne, M13mp19, and mp19 were confirmed by restriction mapping. This experiment was recorded on pages 20-21 of notebook 3966. A copy thereof is attached as Exhibit 13.

On or about March 1, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. Restriction analysis was performed on M13 DNA and the clones that were transformed on February 23, 1995, to confirm the identities of the constructs. This activity was recorded on pages 22-23 of notebook 3966. A copy thereof is attached as Exhibit 14.

On or about March 2, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. The products of the restriction reactions performed on March 1, 1995, were purified by agarose gel electrophoresis, and the appropriate fragments were excised from the agarose gel and purified. The excised *Tne* polymerase gene fragment was ligated into M13 and host cells were

transformed. This experiment was recorded on pages 23-24 of notebook 3966. A copy thereof is attached as Exhibit 15.

On or about March 7, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the experiment to clone a *Tne* gene fragment into M13. The new M13-*Tne* construct ligated on March 2, 1995 was purified, and the structure of the new construct was confirmed by restriction mapping. This experiment was recorded on pages 25-27 of notebook 3966. A copy thereof is attached as Exhibit 16.

On or about March 8, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, performed an experiment to mutate *Tne* DNA polymerase. Site directed mutagenesis reactions were performed on the new *Tne*-M13 construct to generate a 3'-5' exonuclease mutant using the 2899 oligonucleotide primers, which were designed by Brian Schmidt (*see* Exhibit S-7). Additionally attempts were made to ligate the *Tne* polymerase fragment into Mp18 and Mp19; both new constructs were used to transform *E. coli* host cells. This experiment was recorded on pages 28-30 of notebook 3966. A copy thereof is attached as Exhibit 17.

On or about March 14, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, performed an experiment to culture *Tne*-Mp19 clones. Samples were analyzed by restriction mapping to confirm the identity of the construct. This experiment was recorded on page 31 of notebook 3966. A copy thereof is attached as Exhibit 18.

On or about March 15, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, conducted an experiment to analyze the fragment pattern from the restriction mapping experiments performed on March 14, 1995. The *Tne*-M13 clone was grown and cells were pelleted and stored at -70° C overnight. This experiment was recorded on pages 32-33 of notebook 3966. A copy thereof is attached as Exhibit 19.

On or about March 16, 1995, Avani Patel, working under supervision by me and Deb K. Chatterjee, continued the previous experiment by purifying the vector from the cells stored on March 15, 1995, and restricted the plasmid for further confirmation of the construct. This experiment was recorded on pages 34-35 of notebook 3966. A copy thereof is attached as Exhibit 20.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or document or any registration resulting therefrom.

Further, declarant sayeth not.

Date: November 21, 2001

Name: Brian Schmidt

Signature: 

## Resume

**Brian J. Schmidt**  
13503 Crispin Way  
Rockville, Maryland 20853  
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**2001                      Biologist   NIH/NHGRI (Bethesda, MD)   301.496.9810   bschmidt@nhgri.nih.gov**

- Lab manager for genotyping lab involved in NIDDM (Type 2 Diabetes).

**1985 – 2001            Staff Scientist   Life Technologies, Inc. (Rockville, MD)**

- Develop new strains of competent *E. coli* in unit of use and 96-well formats: **STBL4** (cells for unstable clones and cDNA libraries), **DH5 $\alpha$ T1** (T1 phage -resistant cells for standard cloning), **DH10BT1** (T1 phage-resistant cells for cDNA and genomic libraries), **DH5 $\alpha$ T1mcrFT** (T1 phage-resistant cells for cDNA and genomic libraries).
- Clone and characterize a novel methylase in the *E. coli* W bacteriophage w $\phi$ .
- Mutagenesis of Moloney-Murine Leukemia Virus Reverse Transcriptase (MMLV-RT) to generate a RNase<sup>-</sup> mutant (**Superscript II**).
- Clone, sequence, and over-express: restriction-modification systems of **XmaIII** and **AluI** (U.S. Patent) and DNA polymerases from thermophilic organisms (*D. tok*, *T. thermatoga*, *T. neopolitana*).
- Develop Yeast Artificial Chromosome (YAC) vectors and optimize large-scale preparation of yeast spheroplasts for use in YAC library construction.
- Technical Services/Training Center (3 years): responsible for the support of over 1,000 Cell/Molecular Biology products, database current literature, teach rDNA courses.
- Clone and characterize Human Papilloma Viruses (**HPV**) for use in a clinical detection system.
- Supervision of summer interns.

**1982 – 1985            Research Associate   Genex Corporation (Rockville, MD)**

- Design and construct expression/secretion vectors for use in expression/secretion proteins in *E. coli* and *Bacillus subtilis* (calf rennin, human serum albumin, and glucose dehydrogenase) and characterize the proteins by Western blot analysis, immunoprecipitation, and pulse-chase experiments.

**1981 – 1982            Microbiologist II   Bethesda Research Laboratories (Gaithersburg, MD)**

- Preparation of primary chick fibroblast, primary duck fibroblast, and human melanoma cells.
- Herpes Virus of Turkey (HVT) assays in primary chick and duck fibroblasts.

**1981 – 1981            Technician I   Flow General Laboratories (M<sup>c</sup>Clean, VA)**

- Large-scale tissue culture of human foreskin (FS-4) cells for use in interferon production.

**1980 – 1981            Laboratory Aide   FDA (Rockville, MD)**

- Tissue culture of CHO and CV-1 cells and UV/IR mutagenesis with HSV-1 and SV40.

## Education

**1987 – 1990            The NIH FAES Graduate School Program (Bethesda, MD)**

Biology of Bacterial Plasmids, Nucleic Acid Structure and Function, 3-D Structure of Biological Molecules.

**1980                    University of Maryland (College Park, MD)**

Molecular Genetics, Medical Virology, and Marine Zoology

**1979                    Wallops Island Marine Science Consortium (Wallops Island, VA)**

Marine Biology, Marine Botany, Coastal Mammology, Field Techniques, Independent Research (IBIS Project - American University)

**1975 – 1979            Bloomsburg State College (Bloomsburg, PA)**

Bachelors of Science Degree. Biology with minor in Chemistry



**Instrumentation Knowledge:** Scintillation counting, spectrophotometry, ultracentrifugation, ABI 3100 capillary electrophoresis, PC and Mac (Word, Excel, Clone Manager).

#### **Analytical Procedures and Techniques**

**DNA:** Small/large-scale DNA purification (plasmid, chromosome, and bacteriophage), restriction endonuclease digestion and analysis, modification enzymes (Ligase, Kinase, Phosphatase, Methylase, Reverse Transcriptase, etc.), radioactive and non-radioactive labeling (nick translation, random priming, and PCR), Southern blot analysis, PCR (degenerate primers), cycle sequencing, oligodeoxynucleotide design and purification (UV shadow), site-directed mutagenesis, construction of genomic libraries.

**RNA:** Isolation from tissues and cultured cells (Trizol), synthesis of RNA from T3/T7 vectors, denaturing gels (glyoxal and formaldehyde), Northern blot analysis.

**Protein:** Immunoprecipitation, pulse-chase labeling, Bradford assay, SDS PAGE and Western blot

**General:** Electrophoresis (agarose, acrylamide, and pulse-field), autoradiography, electroporation of bacterial cells

#### **Patents:**

Smith, M. D., Schmidt, B. J., Longo, M. C., Chatterjee, D. K. (1994) Cloning and Expression of AluI Restriction Endonuclease. U.S. Patent # 5,334,526

Bloom, F.R., Schmidt, B. J., Lin, J. J. (2001) Rapid Growing Microorganisms (U. S. Patent Pending)

#### **Publications:**

1. Schmidt, B. J. and Bloom, F. R. (1999) ElectroMax STBL4 for Stable Maintenance of Repeat Sequences. *FOCUS* 21, 3.
2. Schmidt, B. J., Natarajan, P., Dube, S., Fox, D., Crouse, J. (1998) Alkaline Phosphatase Technical Bulletin.
3. Schmidt, B. J., Natarajan, P., Fox, D. (1998) Use of Alkaline Phosphatases in Cloning. *FOCUS* 20, 2.
4. Li, W. B., Gruber, C., Noon, M.C., Polayes, D., Schmidt, B. J., Jesse, J. (1995) The Rapid Isolation of Specific Genes from cDNA Libraries with the GeneTrapper Positive Selection System. *FOCUS*, 17, 2.
5. Gerard, G. F., Schmidt, B. J., Kotewicz, M. L., Campbell, J. C. (1992) cDNA Synthesis by Moloney Murine Leukemia Virus RNaseH<sup>-</sup> Reverse Transcriptase Possessing Full DNA Polymerase Activity. *FOCUS* 14, 3.
6. Lorincz, A., Quinn, A. P., Goldsborough, M., D., Schmidt, B. J., Temple, G. F. (1989) Cloning and Partial DNA Sequencing of Two New Human Papilloma Virus Types Associated with Condylomas and Low-Grade Neoplasia. *J. of Virology* 6:2829-2835.
7. Saunders, C. W., Schmidt, B. J., Mallonee, R. L., Guyer, M. S. (1987) Secretion of Human Serum Albumin from *Bacillus subtilis*. *J. of Bacteriology*, 7:2917-2925.
8. Schmidt, B. J., Strasser, J., Saunders, C. W. (1986) A *Bacillus subtilis* Plasmid That Can be Packaged as Single-Stranded DNA in *Escherichia coli*. *Gene*, 41:331-335.
9. Rudolph, C. J., Schmidt, B. J., Saunders, C. W. (1986) Transformation of *B. subtilis* by Single-Stranded DNA. *J. of Bacteriology*, 3:1015-1018.
10. Smith, M. D., Flickinger, J. F., Lineberger, D. W., Schmidt, B. J. (1986) Protoplast Transformation in Coryneform Bacteria and the Introduction of  $\alpha$ -amylase from *Bacillus amyloliquefaciens* in *Brevibacterium lactofermentum*. *Applied and Environmental Microbiology*, 3:634-639.
11. Chaudry, G. R., Halpern, Y. S., Saunders, C. W., Vasantha, N., Schmidt, B. J., and Freese, E. (1984) Mapping of the Glucose Dehydrogenase Gene in *Bacillus subtilis*. *J. of Bacteriology*, 160:607-611,
12. Saunders, C. W., Schmidt, B., Mirot, M., Thompson, L., Guyer, M. (1984) The Use of Chromosomal Integration in the Establishment and Expression of *blaZ*, a *Staphylococcus aureus*  $\beta$ -lactamase Gene in *Bacillus subtilis*. *J. of Bacteriology*, 157:718-726.

13. Saunders, C. W., Banner, C. D., Fahnestock, S. R., Lindberg, M., Mirot, M. W., Rhodes, C. S., Rudolph, C. R., **Schmidt, B. J.**, Thompson, L. D., Uklen, M., Guyer, M.S. (1984) Use of Chromosomal Integration for the Establishment of Heterologous Genes in *Bacillus subtilis*. In "Protein Transport and Secretion". Alan R. Liss, New York. pp. 329-339
14. Bockstahler, L.E., Coohill, T. P., Lytle, C. D., Moore, S. P., Cantwell, J. M., **Schmidt, B. J.**, (1982) Tumor Virus Induction and Host Cell Capacity Inactivation – Possible *in vitro* Tests for Photosensitizing Chemicals. J. NCI, 1:183-188.
15. Lytle, C. D. and **Schmidt, B. J.** (1981) Host Cell Reactivation and UV-Enhanced Reactivation in Synchronized Mammalian Cells. Photochemistry and Photobiology. 34:355-359

References available upon request.